

10/528218
PCT/CA2003/001429**CLAIMS:**

1. An analyzer for simultaneously detecting and measuring the concentration of two related analytes, said analytes being substrates for a common enzyme, comprising:

(a) an enzymatic reaction monitoring component including a support base, a mixed electrode system consisting of a working electrode, an auxiliary electrode and a reference electrode, said mixed electrode system being supported by said support base, and an enzymatic reaction means incorporating said enzyme, said enzymatic reaction means being disposed on said mixed electrode system; whereby, when said enzymatic reaction means is placed in contact with a liquid sample containing said two related analytes, said two related analytes chemically react with said enzyme to produce an electronic signal directly related to the concentration of each of said two related analytes in said liquid sample;

(b) a detector including a sensor, said detector being connected to said enzymatic reaction monitoring component and capable of continuously detecting and amplifying said electronic signal to produce amplified signals; and

(c) a data processor capable of converting the amplified signals into numerical data representative of the concentration of each of said two related analytes.

2. An analyzer as defined in claim 1, wherein said working electrode and said auxiliary electrode are composed of platinum, and wherein said reference electrode is composed of silver.

3. An analyzer as defined in claim 2, wherein said enzymatic reaction means comprises a layer of a permeable polymer on which is bound a

layer including said enzyme, said layer being deposited on said mixed electrode system, and a protective membrane impregnable with a buffer solution and reagents capable of promoting said enzymatic reaction, said protective membrane being disposed over said layer of a permeable polymer.

4. An analyzer as defined in claim 3, wherein said permeable polymer is selected from the group consisting of polylysine, poly(4-styrene sulfonate), polyethylene glycol, perfluorosulfonic acid polymers and agarose.

5. An analyzer as defined in claim 4, wherein said reagents include electron transfer reagents selected from the group consisting of p-phenylenediamine, peroxidase and ferrocene derivatives.

6. An analyzer as defined in claim 5, wherein said ferrocene derivatives include ferrocene dicarboxylic acid, and ferrocene monocarboxylic acid.

7. An analyzer as defined in claim 6, wherein said buffer solution is selected from the group consisting of phosphates, saline phosphate buffers (phosphates + NaCl), TRIS-HCl, Hepes, with or without EDTA, and a wetting agent such as SDS, Triton X-100 and Tween 20.

8. An analyser as defined in claim 7, wherein said enzymatic reaction monitoring component is a disposable electrode.

9. An analyzer as defined in claim 2, wherein said enzymatic reaction means comprises a reagent well capable of receiving a buffer solution including said enzyme, said liquid sample, and optionally reagents capable of promoting said enzymatic reaction.

10. An analyzer as defined in claim 9, wherein said reagents include electron transfer reagents selected from the group consisting of p-phenylenediamine, peroxidase and ferrocene derivatives.

11. An analyzer as defined in claim 10, wherein said ferrocene derivatives include ferrocene dicarboxylic acid, and ferrocene monocarboxylic acid.

12. An analyzer as defined in claim 11, wherein said buffer solution is selected from the group consisting of phosphates, saline phosphate buffers (phosphates + NaCl), TRIS-HCl, Hepes, with or without EDTA, and a wetting agent such as SDS, Triton X-100 and Tween 20.

13. An analyser as defined in claim 12, wherein said enzymatic reaction monitoring component is a permanent electrode.

14. An analyzer as defined in claims 8 and 13, wherein said enzyme is an oxidase.

15. An analyser as defined in claim 14, wherein said oxidase is alcohol oxidase.

16. An analyzer as defined in claim 15, wherein said related analytes are methanol and ethanol.

17. An analyzer as defined in claim 16, wherein said liquid sample is a biological specimen selected from the group consisting of saliva, blood or serum.

18. An analyzer as defined in claim 17, wherein said support base is composed of any suitable material capable of supporting said mixed electrode system.

19. An analyzer as defined in claim 18, wherein said support base is composed of plastic.

20. An analyzer as defined in claim 8, wherein said analyzer is a portable analyzer.

21. An analyzer as defined in claim 13, wherein said analyzer is a non-portable analyzer.

22. An analyzer as defined in claims 20 and 21, for use in point-of-care units, in laboratories, in police services, in forensic applications and in industrial applications.

23. A method for simultaneously detecting and measuring the concentration of at least two related analytes in a sample, said related analytes being substrates for a common enzyme, wherein said enzyme reacts with said related analytes following specific different reaction kinetics, and wherein said method comprises:

(a) reacting a plurality of reference samples having known concentrations and proportions of said related analytes, said proportions ranging from 0 to 100% of a first analyte to 100% to 0% of another related analyte, with said enzyme;

(b) establishing a kinetic profile having at least two points for each of said plurality of reference samples; and

(c) reacting a test sample comprising an unknown concentration and proportion of said related analytes with said enzyme and determining the concentration of said related compounds in said test sample using said established kinetic profiles.

24. A method as defined in claim 23, wherein said unknown concentration of said related analytes is established using multiple regression analysis of said kinetic profile.

25. A method as defined in claim 23, wherein said unknown concentration of said related analytes is established using reaction kinetics equations.

26. A method as defined in claim 24 and 25, wherein said related analytes are methanol and ethanol.

27. An enzymatic reaction monitoring component for simultaneously detecting and measuring the concentration of two related analytes, said analytes being substrates for a common enzyme, comprising:

- (a) a support base;
- (b) a mixed electrode system consisting of a working electrode, an auxiliary electrode and a reference electrode, said mixed electrode system being supported by said support base; and
- (c) an enzymatic reaction means incorporating said enzyme, said enzymatic reaction means being disposed on said mixed electrode system; whereby, when said enzymatic reaction means is placed in contact with a liquid sample containing said two related analytes, said two related analytes chemically

react with said enzyme to produce an electronic signal directly related to the concentration of each of said two related analytes in said liquid sample.

28. An enzymatic reaction monitoring component as defined in claim 27, wherein said working electrode and said auxiliary electrode are composed of platinum, and wherein said reference electrode is composed of silver.

29. An enzymatic reaction monitoring component as defined in claim 28, wherein said enzymatic reaction means comprises a layer of a permeable polymer on which is bound an enzyme layer, said enzyme layer being deposited on said mixed electrode system, and a protective membrane impregnable with a buffer solution and reagents capable of promoting said enzymatic reaction, said protective membrane being disposed over said layer of a permeable polymer.

30. An enzymatic reaction monitoring component as defined in claim 29, wherein said permeable polymer is selected from the group consisting of polylysine, poly(4-styrene sulfonate), polyethylene glycol, perfluorosulfonic acid polymers and agarose.

31. An enzymatic reaction monitoring component as defined in claim 30, wherein said reagents include electron transfer reagents selected from the group consisting of p-phenylenediamine, peroxidase and ferrocene derivatives.

32. An enzymatic reaction monitoring component as defined in claim 31, wherein said ferrocene derivatives include ferrocene dicarboxylic acid, and ferrocene monocarboxylic acid.

33. An enzymatic reaction monitoring component as defined in claim 32, wherein said buffer solution is selected from the group consisting of phosphates, saline phosphate buffers (phosphates + NaCl), TRIS-HCl, Hepes, with or without EDTA, and a wetting agent such as SDS, Triton X-100 and Tween 20.

34. An enzymatic reaction monitoring component as defined in claim 33, wherein said enzymatic reaction monitoring component is a disposable electrode.

35. An enzymatic reaction monitoring component as defined in claim 28, wherein enzymatic reaction means comprises a reagent well capable of receiving a buffer solution including said enzyme, said liquid sample, and optionally reagents capable of promoting said enzymatic reaction.

36. An enzymatic reaction monitoring component as defined in claim 35, wherein said reagents include electron transfer reagents selected from the group consisting of p-phenylenediamine, peroxidase and ferrocene derivatives.

37. An enzymatic reaction monitoring component as defined in claim 36, wherein said ferrocene derivatives include ferrocene dicarboxylic acid, and ferrocene monocarboxylic acid.

38. An enzymatic reaction monitoring component as defined in claim 37, wherein said buffer solution is selected from the group consisting of phosphates, saline phosphate buffers (phosphates + NaCl), TRIS-HCl, Hepes, with or without EDTA, and a wetting agent such as SDS, Triton X-100 and Tween 20.

39. An enzymatic reaction monitoring component as defined in claim 38, wherein said enzymatic reaction monitoring component is a permanent electrode.

40. An enzymatic reaction monitoring component as defined in claims 34 and 39, wherein said enzyme is an oxidase.

41. An enzymatic reaction monitoring component as defined in claim 40, wherein said oxidase is alcohol oxidase.

42. An enzymatic reaction monitoring component as defined in claim 41, wherein said related analytes are methanol and ethanol.

43. An enzymatic reaction monitoring component as defined in claim 42, wherein said liquid sample is a biological specimen selected from the group consisting of saliva, blood or serum.

44. An enzymatic reaction monitoring component as defined in claim 43, wherein said support base is composed of any suitable material capable of supporting said mixed electrode system.

45. An enzymatic reaction monitoring component as defined in claim 44, wherein said support base is composed of plastic.